Distribution of Functional Glutamate and GABA Receptors on Hippocampal Pyramidal Cells and Interneurons

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Pettit, Diana L. and George J. Augustine. Distribution of functional glutamate and GABA receptors on hippocampal pyramidal cells and interneurons. J Neurophysiol 84: 28–38, 2000. The distribution of functional neurotransmitter receptors is an important determinant of neuronal information processing. To map the location of functional glutamate and GABA receptors on individual hippocampal neurons, we photolyzed “caged” glutamate and GABA while measuring the electrical currents resulting from activation of these receptors. Responses to uncaged neurotransmitters were spatially nonuniform and varied according to the type of receptor and type of neuron. Every region of CA1 pyramidal cells responded to glutamate and GABA, but glutamate and GABA receptors increased in density along the length of their distal dendrites. Similar gradients of glutamate receptors were found in stratum radiatum interneurons, while GABA responses were detectable only in the perisomatic region of these interneurons. These regional variations in receptor distribution indicate the selective targeting of receptors on central neurons and may reflect a mechanism for local regulation of synaptic efficacy.

INTRODUCTION

Synaptic transmission requires the specific targeting of neurotransmitter receptors to defined domains in the plasma membrane of postsynaptic cells. While targeting of neurotransmitter receptors has been documented in some detail at peripheral synapses (Hall and Sanes 1993), much less is known about the targeting of neurotransmitter receptors at central synapses (Craig et al. 1994; Gomperts et al. 1998; Liao et al. 1999; Mammen et al. 1997; Xia et al. 1999). The first step in approaching this question is to determine where these receptors are found. This issue has been approached by using immunocytochemistry to localize receptors in fixed tissue (Baude et al. 1995; Craig et al. 1994; Landsend et al. 1997). However, such experiments do not provide information about the density of functional receptors because they generally do not discriminate between receptors on the plasma membrane and other sites of antibody binding. Ionophoretic application of transmitter can examine the distribution of functional receptors in certain cases, such as cultured neurons (e.g., Trussell et al. 1988), but geometric constraints limit access to receptors in more intact systems, such as brain slices.

Here we use localized photolysis of caged neurotransmitters (Denk 1994; Gee et al. 1994; Hess 1993; Pettit et al. 1997) to determine the distribution of functional glutamate and γ-aminobutyric acid (GABA) receptors on CA1 pyramidal cells and stratum radiatum interneurons in hippocampal slices. We have found that these receptors are targeted to specific regions along the dendrites and cell bodies in patterns that differ in these two types of neurons. For pyramidal cells, both glutamate and GABA receptors increase in density toward the periphery of dendrites. In contrast, GABA and glutamate receptors have inverse gradients in the interneurons; while glutamate receptors increase along dendrites, GABA receptors decline. This heterogeneity in receptor density may reflect a mechanism for regional regulation of synaptic function.

METHODS

Animals

Standard techniques were used to prepare 400-μm-thick slices from the hippocampus of 13–19 day old rats (Dingledine 1984) and to make whole cell patch-clamp recordings from CA1 pyramidal cells or stratum radiatum interneurons. Pipettes were filled with a solution that contained (in mM) 100 gluconic acid, 2–10 EGTA, 5 MgCl2, 2 Mg-ATP, 0.3 GTP, and 40 HEPES, pH to 7.2 with CsOH. In some experiments, 0.5% biocytin (biotinoyl-L-lysine, Molecular Probes, Eugene, OR) was added to this solution for histological examination after the experiment ended. Slices were superfused at 21°C with oxygenated physiological saline (in mM: 119 NaCl, 2.5 KCl, 1.3 MgCl2, 2.5 CaCl2, 1 NaH2PO4, 26.2 NaHCO3, and 11 glucose) containing 1 μM tetrodotoxin to prevent activation of synaptic transmission via photostimulation (Callaway and Katz 1993; Pettit et al. 1999) and either double-caged glutamate (200 μM, in 0.01% DMSO) or single-caged GABA (100 μM; Molecular Probes, Eugene, OR). Recordings were accepted only if the holding current was <100 pA when pyramidal cells were voltage clamped at −60 mV or when interneurons were held at −70 mV.

Mapping

The 351- to 364-nm output of a continuous emission 8W argon ion laser (Coherent model 305) was delivered, via a multimode optical fiber, through an Olympus ×40 water-immersion objective (Wang and Augustine 1995). At the specimen focal plane, the ultraviolet (UV) light formed a gaussian spot 4.6 μm wide at half-maximal intensity. Light intensity at the front of the objective was 0.1–4 mW, and an electronic shutter (Uniblitz) was used to vary the duration of the light pulse (3–10 ms). The uncaging spot was positioned over a cellular process by including a fluorescent dye (Cadium Green-1 or Oregon BAPTA Green, 200 μM; Molecular Probes, Eugene OR) in the patch pipette solution and then visualizing the cell with a real-time...
confocal microscope (Noran Odyssey XL). To avoid possible phototoxic effects, illumination was kept to a minimum. Following mapping, cells were allowed to fill for 30 min, and the dye-filled cell was optically sectioned with the confocal microscope. These images were then rendered off-line into a three-dimensional reconstruction that was projected into two dimensions to yield the images shown in Figs. 1, 3, 4, 6, and 8. To ensure that small, out of focus, dendrites were not included within the uncaging area, each dendrite was scanned in the axial direction. In none of the cells described here did we observe multiple response peaks in the axial direction, as is the case when more than one dendrite is located in the uncaging area (Pettit et al. 1997). In addition, the rendered cell image was rotated 90° and viewed on its side, and no additional processes were observed. Finally, to exclude the possibility that very fine dendrites, invisible in fluorescent images, were present in the uncaging area, cells were filled with biocytin, and these images were compared with their fluorescence images. No additional processes were identified in the biocytin-filled neurons, and in fact it was easier to identify small processes in the fluorescence images (Fig. 3A).

To measure responses to uncaged glutamate, CA1 pyramidal cells were held at −60 mV and interneurons were held at −70 mV. The dendrite was positioned at the focal point of the uncaging beam by focusing up and down until a maximal response was achieved. Five light flashes were delivered to each spot at 5-s intervals, acquired with Pclamp software and analyzed with Clampfit software (both from Axon Instruments, Foster City, CA). Responses at a given location were highly reproducible, with intertrial variability of <5%. Unless specified, all experiments were done in the presence of 50 μM cadmium to block calcium influx. To examine responses to uncaged GABA, cells were depolarized to −30 mV, and uncaging pulses were delivered at 10- to 15-s intervals. Current-voltage relationships were determined by repeated GABA uncaging at a single location while varying the holding potential at 10-mV intervals.

The diameter of the dendrite under each uncaging spot was determined by measuring the fluorescence intensity for individual voxels across the width of the dendrite in reconstructed cell images. The half-maximal width of this distribution was then used as a measure of diameter. Uncaging locations whose fluorescence intensity scans did not have a simple, gaussian profile were not analyzed. The surface area over which transmitter was uncaged was calculated by assuming that the dendrite was cylindrical and that the transmitter was generated along a length equal to the size of the uncaging spot (4.6 μm).

FIG. 1. Neurotransmitter responses of a CA1 pyramidal neuron. A: the lateral resolution of photolysis was determined by scanning the ultraviolet (UV) light beam across a dendrite at 1.5-μm intervals in the presence of caged GABA (○) and caged glutamate (●). Gray bar represents the location of the dendrite. B, left: superimposition of currents elicited by 5 glutamate applications over a distal dendrite. UV light was applied during the times indicated by the bar. These glutamate responses were variable in time course, and their decay was irregular due to a 2nd current component that was blocked by cadmium (right). C: a volume-rendered image of a CA1 pyramidal neuron filled with fluorescent dye via a patch pipette (at left). Circles indicate the position and diameter of the UV light spot used. Traces at right are averages of 5 responses evoked from the locations indicated by the numbers. Traces 1–3 are responses from basal dendrites; traces 6–9 are responses from apical dendrites. Moving the uncaging spot off the neuron elicited no glutamate response from the neuron (trace 5).
Biocytin-filled neurons were fixed in paraformaldehyde, dehydrated and cleared following a dianaminobenzidine reaction. The filled cells were photographed, and their images were scanned into the computer. Measurements of pixel intensity under the uncaging spots were compared with those obtained from the optical images. Measurements were corrected for shrinkage by comparison to the live optical images on a slice-by-slice basis and were between 30 and 40%. Before correction, diameters of biocytin-filled processes were smaller than those of the fluorescence images but followed the same distribution; after this correction the two measurements were nearly identical.

To calculate the surface area of the cell body exposed to uncaged transmitters, the cell body was assumed to be a sphere 20 \( \mu m \) in diameter centered in the uncaging beam. For double-caged glutamate, the exposed surface was then two circles with diameters equal to the diameter of the uncaging cone, for a total area of 125 \( \mu m^2 \). The volume over which single-caged GABA is photolyzed should be much larger (Pettit et al. 1997), and our calculations predict that the somatic surface area exposed to free GABA was 498 \( \mu m^2 \).

**RESULTS**

Whole cell patch-clamp recordings were made from the cell bodies of neurons in hippocampal slices while simultaneously scanning a UV light spot along the neuron to generate active neurotransmitter over a restricted region of these neurons. The spot had a gaussian distribution of light and was 4.6 \( \mu m \) in diameter at half-maximal intensity (Wang and Augustine 1995). By measuring electrical currents resulting from placing the UV light spot at various positions, it was possible to map regional variations in the transmitter responsiveness of individual neurons. A fluorescent dye, either Calcium Green-1 or Oregon BAPTA Green-1, was included in the recording pipette solution to visualize the processes of these cells during the physiological measurements. To avoid phototoxicity, we reduced the intensity of the fluorescence excitation light and were still able to visualize all but the most distal regions of dendrites. We were routinely able to position the light spot as far as 250 \( \mu m \) along apical dendrites and up to 100 \( \mu m \) along basal dendrites. After the physiological measurements were completed, the intensity of the fluorescence excitation light was increased to obtain a high-resolution, three-dimensional image of the cell with a confocal microscope. This approach was used to examine the responsiveness of both CA1 pyramidal neurons and stratum radiatum interneurons to glutamate and GABA.

A double-caged glutamate compound was used to obtain optimal spatial resolution when examining glutamate receptors (Pettit et al. 1997). The half-width of glutamate application, determined by scanning the UV light spot across a pyramidal cell dendrite, was 5 \( \mu m \), approximately the same diameter as the light spot (Fig. 1A). As a result, either no glutamate responses or very small responses were observed when the spot was displaced 5 \( \mu m \) away from the dendrite (for example, trace 5 in Fig. 1C). Because no double-caged GABA compound has been successfully synthesized, we used single-caged GABA (Wang and Augustine 1995) to determine the distribution of GABA receptors. As expected (Pettit et al. 1997), the lateral resolution of GABA delivery was inferior with this single-caged compound. The half-width of GABA application was on the order of 10 \( \mu m \) (Fig. 1A), approximately two times poorer than that determined for the glutamate on the same dendrite. The presence of two caging groups should also make the axial resolution for glutamate application superior to that of GABA (Pettit et al. 1997; Wang et al. 2000).

**Mapping glutamate responsiveness of pyramidal neurons**

Photolysis of double-caged glutamate evoked inward currents in all pyramidal neurons examined (Fig. 1B). These currents were detected in the presence of tetrodotoxin (1 \( \mu M \)), to block voltage-gated sodium channels, and cesium in the recording pipette to block voltage-gated potassium channels. Uncaging of glutamate over the pyramidal cell dendrites, particularly over dendritic branch points, often elicited a secondary inward current component (Fig. 1B, left). The second component was blocked by bath application of 50 \( \mu M \) cadmium (Fig. 1B, right), suggesting that this component arose from activation of voltage-gated calcium channels. To avoid this complication, all mapping experiments were done in the presence of cadmium.

Currents elicited in pyramidal neurons by photolysis of double-caged glutamate were caused by activation of glutamate receptors because they were entirely abolished (not shown) (see Pettit et al. 1997) by treatment with 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 10 \( \mu M \)), an antagonist of \( \alpha \)-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)–type glutamate receptors (Honore et al. 1988), in combination with 2-amino-5-phosphonovaric acid (APV; 100 \( \mu M \)), which blocks N-methyl-D-aspartate (NMDA)–type glutamate receptors (Watkins and Collingridge 1989). APV had no effect on the peak amplitude of the responses evoked by uncaged glutamate but did eliminate a slowly decaying current component that had a time constant of 51 \pm 6.9 ms (Pettit et al. 1997). The more rapidly decaying component, produced by AMPA receptors, had a time constant of 14 \pm 1.1 ms (n = 12). Thus the pyramidal cell glutamate responses were caused by activation of both AMPA-type and NMDA-type glutamate receptors.

**Glutamate receptor gradients on pyramidal neurons**

Responses to double-caged glutamate could be observed when glutamate was generated over any region of CA1 pyramidal neurons (Fig. 1C). Surprisingly, substantial currents could be elicited from the cell body (Fig. 1C, trace 4) despite the fact that very few excitatory synapses occur at the soma (Muller et al. 1984); thus pyramidal cell somata contain large numbers of extrasynaptic receptors. In all pyramidal neurons examined, the amplitude of glutamate responses varied in a consistent pattern along the length of the dendritic processes. In basal dendrites, the amplitude of glutamate responses decreased rapidly as the light spot was moved further from the cell body (Fig. 1C, trace 2). In contrast, apical dendrite responses had a very different spatial pattern. When the uncaging spot was moved to the primary apical dendrite (Fig. 1C, trace 6), the amplitude of glutamate responses decreased. However, when the uncaging spot was moved even further away, the amplitude of the glutamate responses increased. For example, in Fig. 1C the glutamate-induced current increased by 50% when the light spot was moved from site 6, a location on the primary apical dendrite, to site 7, 30 \( \mu m \) beyond the first branch in the dendrite. The time courses of these responses did not change significantly as the glutamate was photoreleased at
more distal locations (Fig. 1C) and the spatial profile of glutamate responses was unaffected by application of APV (100 μM), indicating that these gradients mainly reflecting the opening of AMPA-type glutamate receptors.

Increases in the glutamate responses along the length of apical dendrites were consistently seen in each of eight experiments that mapped glutamate responses on CA1 pyramidal neurons (Fig. 2A). Glutamate response amplitude seemed to vary, at least in part, according to the location of the glutamate application relative to dendritic branch points. When plotted as a function of the order of the dendritic branch, it could be seen that the increase in glutamate current amplitude along the apical dendrite was restricted to the secondary and tertiary dendrites (Fig. 2B; P < 0.001 with ANOVA).

While the UV light spot had a constant diameter, the diameter of dendrites varied and different amounts of plasma membrane were exposed to uncaged glutamate as the spot was scanned along the dendrite (Fig. 1C). To account for such variations in surface area, we used volume-rendered confocal images of the pyramidal neurons and the known geometry of the UV light beam (Pettit et al. 1997; Wang and Augustine 1995) to estimate, for each uncaging site, the area of plasma membrane over which glutamate was released. The diameter of dendritic processes under the UV light spot was first quantified by measuring the profile of fluorescence intensity across the width of the process (Fig. 3, A and B). The average diameters of 2.3 ± 0.1 (SE) μm for primary dendrites, 0.8 ± 0.1 μm for secondary dendrites, and 0.6 ± 0.1 μm for tertiary dendrites were very similar to values of 2.7 μm reported for the diameter of primary dendrites and 0.8, 0.68, and 0.64 μm reported for secondary and tertiary dendrites (Bannister and Larkman 1995). To confirm the fidelity of our fluorescence measurements, in five experiments biocytin was also included in the solution as an independent means of visualizing dendrites (Fig. 3A, right). Measurements of the diameters of dendrites filled with biocytin were in excellent agreement with measurements made from the same dendrites in fluorescence images (Fig. 3A, left). When the diameters of dendritic individual uncaging sites were compared, the mean difference in the values obtained by the two methods was insignificant (0.1 ± 0.06 μm; n = 15; P > 0.25, paired Student’s t-test). The close agreement of the measurements indicates that either method could be used to quantify dendritic size; we routinely used the fluorescence procedure because it was simpler and allowed us to obtain images obtained from live, unfixed neurons. Membrane surface areas were next calculated from our estimates of dendritic diameter, and the density of glutamate-induced electrical current at each site was then determined by dividing the peak amplitude of each current by the area exposed to glutamate.

The density of glutamate-induced currents (Fig. 3C) was even less spatially uniform than the amplitude of these currents (Fig. 2, A and B). Because of the larger active area of the cell body, current density was higher in the dendrites than in the cell body, and highest in the smallest, most distal dendrites (Fig. 3C). Although current amplitude declined along basal

FIG. 2. Nonuniformity of glutamate responses on pyramidal neurons. A: relationship between the location of the UV light spot with respect to the soma and the amplitude of the current induced by glutamate at each location. The peak amplitude of the glutamate-induced currents has been normalized to the amplitude of currents evoked over the cell body of each of the 8 neurons examined. B: the same glutamate responses shown in A, considered as a function of dendritic branch.

FIG. 3. Determination of glutamate response density. A: images of the same cell filled with a fluorescent dye (left) and biocytin (right). Arrows indicate the location and direction of intensity measurements shown in B. B: measurements of dendritic diameter reported by fluorescence image (top) and biocytin imager (middle). Superimposition of these 2 measurements (bottom) indicates that the dendrite is 1.4 μm wide. C: relationship between the location of the UV light spot and the mean density of the current induced by glutamate at each location, calculated by dividing current amplitude by the area over which glutamate was photoreleased (n = 5).
dendrites (Fig. 2, A and B), the area of these dendrites declined more steeply so that there was a two- to threefold higher density of glutamate-induced current in their distal regions (Fig. 3C). The gradients in current density were even more dramatic in the apical dendrites, with six- to sevenfold higher current densities in the secondary and tertiary dendrites compared with the primary dendrite or cell body (Fig. 3C). In summary, these data indicate that the spatial gradients of glutamate responsiveness are not due to differences in the area over which glutamate was applied; on the contrary, the distally directed gradients were amplified after accounting for these differences in areas. Arguments presented in the DISCUSSION indicate that these and other gradients described in this paper cannot be explained by poor spatial control of membrane potential. We therefore conclude that glutamate receptors, specifically the AMPA-type glutamate receptor, distribute heterogeneously over the surface of pyramidal neurons.

Nonuniform GABA responsiveness of pyramidal neurons

The distribution of functional synaptic and extrasynaptic GABA receptors was determined in experimental conditions that caused GABA receptors to produce an outward current when activated. Under these conditions (129 mM external chloride; 10 mM internal chloride), the reversal potential for GABA-evoked chloride currents was calculated to be −64 mV. GABA mapping experiments therefore were performed at a membrane potential of −30 mV to provide driving force for chloride influx. While the 36-mV difference between the chloride equilibrium potential and the holding potential could allow small nonuniformities in control of dendritic membrane potential to influence the magnitude of dendritic GABA responses, this did not appear to occur because currents evoked by GABA at distal dendrites reversed at the same potential as those evoked by GABA application at the soma (Fig. 4A). The average reversal potential for GABA photoreleased over the cell body was −63 ± 2.6 mV (n = 6), −64 ± 1.6 mV (n = 6) for responses of the tertiary apical dendrites, and −64 ± 1.8 mV (n = 4) for responses of the tertiary basal dendrites (ANOVA; P > 0.75). These results indicate a constant driving force for chloride ions along the length of pyramidal neurons.

Responses to uncaged GABA were observed in all regions of pyramidal neurons. These responses were due to activation of GABA_A receptors because they were completely blocked by picrotoxin (100 μM; data not shown), a GABA_A receptor antagonist (Yoon et al. 1993). No responses mediated by GABA_B receptors were detected, presumably due to the presence of the potassium channel blocker, cesium, in the internal solution. The decay of these currents could be described by a single exponential function with a mean time constant of 140 ± 12 ms (n = 5). Similar slow responses following GABA uncaging also have been observed in cerebellar Purkinje cells (Wang and Augustine 1995); the slow decay of these currents in comparison to glutamate-induced currents presumably is due to the slow rate of extracellular GABA removal. GABA responses decayed somewhat more slowly in the dendrites than in the cell body in the example shown in Fig. 4, but this was not consistently observed in other experiments.

The amplitude of GABA responses varied along the length of the dendritic processes in a pattern qualitatively similar to what we observed for glutamate responses (Fig. 4B). GABA responses decreased in amplitude with increasing distance from the cell body along the basal dendrites (Fig. 4B, traces 3–1). As the uncaging spot was moved from the cell body to the primary apical dendrite, GABA responses remained approximately constant in amplitude (Fig. 4B, traces 4 and 6). Results from six experiments in which GABA responses were evoked along the apical and basal dendrites of CA1 pyramidal neurons are summarized in Fig. 5A. GABA responses decreased monotonically with distance along the basal dendrites of pyramidal cells, while the amplitude of these responses

FIG. 4. GABA responses of a CA1 pyramidal neuron. A: relationship between somatic membrane potential and the peak amplitude of current evoked by photolyzing caged GABA over the cell body, tertiary apical dendrite and basal dendrite of a pyramidal cell. B: a volume-rendered image of a CA1 pyramidal neuron filled with fluorescent dye via a patch pipette (at right). Circles indicate the position and diameter of the UV light spot used to uncage GABA. Traces at right are averages of 3 responses evoked by uncaging GABA. Traces 1 and 2 are responses from basal dendrites, while traces 4–9 are responses from apical dendrites.
remained fairly constant along the length of apical dendrites. A very similar trend was detected when GABA current amplitude was plotted as a function of dendritic branch (Fig. 5B). To account for differences in the surface area over which GABA was applied, GABA current density was calculated as described above for glutamate currents. The highest densities of GABA-induced currents occurred in the distal regions of both the apical and basal dendritic trees (Fig. 5C). GABA current density was approximately twofold higher in the distal branches of basal dendrites than in the primary basal dendrites, and approximately three to four times higher in distal apical dendrites than in primary apical dendrites. An ANOVA showed that differences in branch density were significant \((P < 0.001)\). The density of GABA-induced currents was lowest on CA1 cell bodies and higher for apical dendrites than for basal dendrites. We conclude that there are regional differences in the distribution of functional GABA receptors in the somata and dendrites of CA1 pyramidal neurons.

**Glutamate receptor gradients in interneurons**

Hippocampal CA1 stratum radiatum interneurons send GABAergic inputs to pyramidal cells and receive some of the same synaptic inputs that innervate pyramidal neurons (Buhl et al. 1994; Gulyas and Freund 1996). We next examined the responsiveness of these interneurons to uncaged glutamate and GABA. Although hippocampal interneurons are quite heterogeneous in their physiological properties (Acsady et al. 1996; Cobb et al. 1997; Sik et al. 1995), the pattern of glutamate responsiveness was consistent for all eight stratum radiatum interneurons.

**FIG. 5.** Nonuniform density of GABA responses on pyramidal neurons. A: relationship between the location of the UV light spot with respect to the cell body and the amplitude of the current induced by GABA at each location. The peak amplitude of the GABA-induced currents have been normalized to the amplitude of currents evoked over the cell body of each of the 6 neurons examined. B: same data as in A plotted with respect to dendritic branch. C: relationship between the location of the UV light spot and the density of the current induced by GABA at each location \((n = 6)\).

**FIG. 6.** Glutamate responses of a CA1 stratum radiatum interneuron. A, left: superimposition of currents elicited by 5 glutamate applications over an interneuron. UV light was applied during the times indicated by the bar. Right: glutamate-induced currents were completely eliminated by 2-amino-5-phosphonovaleric acid (APV; 100 \(\mu\)M) and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 10 \(\mu\)M). B: a volume-rendered image of a dye-filled interneuron. Circles indicate the position and diameter of the UV light spot used to uncage glutamate and traces indicate averages of 5 evoked responses at each location.
CA1 interneurons that we examined. These interneurons were classified by their dendritic branching pattern, as proposed by Parra et al. (1998). Seven of these cells had a stellate dendritic branching pattern, and the eighth had a vertical branching pattern. For each interneuron, electrical currents were detected when double-caged glutamate was photolyzed over them. These currents were blocked by treatment with CNQX and APV, indicating that they are due to activation of glutamate receptors on these interneurons (Fig. 6A). The decay time constants for these somatic glutamate responses were similar to those calculated for pyramidal cells, with means of 12 ± 2.3 ms and 82 ± 13 ms (n = 5). Glutamate-induced currents were slightly faster for interneuron dendrites than for pyramidal cell dendrites, with decay time constants of 7.0 ± 0.8 ms and 81 ± 29 ms (n = 5).

The distribution of the interneuronal glutamate responses was nonuniform, with a spatial pattern differing somewhat from that seen in pyramidal cells. Electrical responses could be detected when glutamate was photoreleased over the cell bodies of interneurons, but when the uncaging spot was moved away from the interneuron soma, glutamate responses only increased in amplitude (Fig. 6B); that is, unlike pyramidal cells, there was no decline in the glutamate response of dendrites close to the cell body. The time courses of these responses did not change significantly as the glutamate was photoreleased at more distal locations. Such increases along the length of the dendrites were observed on all dendrites within a given interneuron; for example, the two processes of the interneuron shown in Fig. 6B produced very similar responses at comparable distances from the cell body.

When comparing glutamate responses from these cells, we plotted response amplitude as a function of distance from the cell body (as in Fig. 2A) because the dendrites of these interneurons did not branch extensively. The absolute amplitudes of glutamate responses of interneuronal dendrites were larger than those of the somata, with dendritic glutamate-induced currents maximally about 150% of those evoked in the cell body (Fig. 7A). This difference was even more striking when the density of glutamate currents was calculated as described above (n = 4), with current density increasing smoothly and steeply as the glutamate was applied to regions farther from the cell body (Fig. 7B). An ANOVA showed that the change in glutamate current density was significant (P < 0.0025). Applying the arguments presented for the glutamate responses of pyramidal cells, we can conclude that interneurons also have a pronounced distally directed gradient of glutamate receptors along their dendrites.

**Inverse GABA receptor gradients on interneuron dendrites**

We also examined the GABA sensitivity of stratum radiatum CA1 interneurons. Our recordings came from four interneurons that had uniform, stellate dendritic branching patterns, apparently identical to the seven interneurons whose glutamate responses were just described. Photolysis of caged GABA over these interneurons elicited outward currents when the cells were held at a potential of -30 mV. These responses were due to activation of GABA_A receptors because they were completely blocked by picrotoxin (Fig. 8A). The decay time constant for interneuronal GABA currents was 130 ± 16 ms (n = 5), similar to the GABA responses of pyramidal cells.

The spatial distribution of functional GABA receptors on these interneurons was markedly different from that of pyra-
trotonic decrement. Thus interneurons appear to concentrate responses in these regions cannot be attributed to dendritic ele-
the distant regions of these cells, the absence of GABA re-
responses could be observed when glutamate was applied to
P distribution that was almost the inverse of that seen for the
5 spot and the density of the current induced by GABA at each location (Fig. 9A; n = 6).

componented cells and from the glutamate responses of these same interneurons. Responses to uncaged GABA were a very sen-
tive function of position and were often undetectable when GABA was photoreleased on dendritic locations only 100 μm
away from the cell body (Fig. 8B). As a result, the spatial profile of interneuronal GABA responses declined steeply with
distance (Fig. 9A; n = 4). Calculations of the density of the GABA-induced current reinforce this conclusion; the density of GABA-induced currents (Fig. 9B; n = 4) showed a spatial distribution that was almost the inverse of that seen for the glutamate responses of these same neurons (Fig. 7B) and were significantly different (ANOVA; P < 0.0075). Given that responses could be observed when glutamate was applied to the distant regions of these cells, the absence of GABA responses in these regions cannot be attributed to dendritic elec-
trotonic decrement. Thus interneurons appear to concentrate their GABA receptors in the perisomatic region.

MID DISCUSSION

We have used local photolysis of caged neurotransmitters to detect regional differences in the distribution of glutamate and GABA receptors on pyramidal cells and interneurons in hip-
locampal slices. We found that responsiveness to these neu-
rotransmitters is not distributed uniformly over the surface of each type of cell and that the distribution of these responses differs for the two types of neurons. CA1 pyramidal neurons have a gradient of glutamate and GABA receptors along their dendrites, with the highest density occurring in the distal regions of both apical and basal dendrites. Our conclusions are consistent with previous anatomical data suggesting that both glutamate and GABA receptors are located along the entire length of the dendrites of these neurons (Baude et al. 1995; Craig et al. 1994; Mammen et al. 1997) and that both excitatory (Baude et al. 1995) and inhibitory (Cobb et al. 1997) synapses may be found along the length of the dendrites. Our results are the first to document a distally directed gradient of functional receptors in hippocampal pyramidal neurons and compliment recent data suggesting a similar arrangement in cortical pyra-
midal cells (Dodt et al. 1998).

While interneurons also have an increasing density of glutamate receptors along their dendrites, these same processes exhibit an inverse gradient of GABA receptors. While we cannot be certain that all functional GABA receptors are associated with synapses, it seems likely that at least some of these GABA receptors are synaptic and provide inhibitory drive to the dendrites (Cobb et al. 1997; Gulyas et al. 1999; Miles et al. 1996). Our findings are consistent with the work of Gulyas et al. (1999), who provided anatomical evidence that the density of CA1 inhibitory interneuron synapses is highest in the perisomatic region. An interesting contrast to our results comes from immunohistochemical reports of GABA receptors distributed along the entire length of interneuronal dendrites (Acsady et al. 1996; Baude et al. 1995; Cobb et al. 1997). A simple explanation for this discrepancy is that receptors rec-
ognized by antibodies may not be functional or even on the plasma membrane. Alternatively, perhaps functional GABA receptors are found in the dendrites of interneurons other than the one type of interneuron that we have examined. At any rate, it is clear that the pattern of targeting of functional GABA receptors varies dramatically among different types of hippo-
campal neurons and along individual neuronal processes.

Mechanisms underlying regional variations in transmitter responsiveness

The spatial variations in GABA and glutamate responses that we observed could arise from a number of different mecha-
isms. In principle, these could be due to imperfect spatial control of membrane potential during our voltage-clamp measure-
ments of transmitter-induced currents. Poor voltage control in the dendrites could have a number of effects on current amplitude. First, this would increase filtering of currents gen-
erated farther away from the cell body, so that distant currents would be preferentially attenuated and slowed. In addition, loss of voltage control with distance could cause an increase in local depolarization and a decrease in the driving force for ions. Both of these effects will yield an underestimate in the magnitude of the observed receptor gradient, so that the effects we describe could be even larger than measured. Finally, loss of voltage control with distance could result in the activation of voltage-dependent channels on the distal dendrites (Magee and Johnston 1995a,b). Our use of pharmacological agents that block voltage-gated sodium, potassium, and calcium channels makes this possibility unlikely. Aside from possible activation of I\(\text{Na}\) channels during GABA responses (Magee 1998), which seems unlikely because GABA-induced currents have a linear voltage dependence (Fig. 4A), any other residual voltage-de-
pendent currents would only cause an underestimation of the response amplitude and an underestimation of response gradi-
ents. In summary, virtually all possible voltage-clamp errors would cause us to underestimate the magnitude of the distally

directed gradients seen for glutamate and GABA in the den-
dendrites of pyramidal cells and for glutamate in interneurons. Thus loss of space clamp cannot account for the changes in transmitter responsiveness we have observed.

It is possible that differences in the unitary conductance and/or affinity of the receptors, rather than the absolute number of these receptors, are responsible for the gradients that we have observed. However, available evidence argues against this interesting possibility; Spruston et al. (1995) found that the unitary properties of glutamate receptors in the cell body and dendrites of CA1 pyramidal neurons were identical. While further studies of the unitary properties of dendritic receptors are warranted, it is unlikely that such differences can account for our results.

Alternatively, variations in neurotransmitter responses could arise from differences in the area of plasma membrane exposed to uncaged neurotransmitters. Although we have attempted to account for this by measuring these areas, our measurements could not take into account dendritic spines because these structures were not clearly resolved in our images. If spine density were higher in distal dendrites than in proximal dendrites, the area of distal dendrite membrane exposed to uncaged neurotransmitter would be systematically underestimated and could give rise to the observed gradient of responses. Of course, this mechanism is unlikely to account for the glutamate receptor gradient observed in interneurons because these neurons typically do not have spines. Further, two considerations argue against the possibility that inequities in spine density account for the receptor gradients observed in pyramidal cells. First, the density of spines is apparently uniform along CA1 pyramidal cell dendrites, except for a lower spine density in the most proximal 20–100 μm region of the primary apical dendrite (Bannister and Larkman 1995; Trommald et al. 1995). Second, any nonuniformities in spine density are quantitatively inadequate to account for most of our results. Estimates suggest 1–3 spines/μm on dendrites of CA1 pyramidal cells (Harris et al. 1992; Ishizuka et al. 1995; Trommald et al. 1995); using the upper estimate of 3 spines/μm and a surface area of 1.15 μm² for a spine (Harris et al. 1992), the area of spines exposed to uncaged glutamate would be about 16 μm². Since the measured surface area of the smallest dendrites was 9 μm², the total area for these dendrites could be as great as 25 μm² after including the spine membrane. This area is still significantly smaller than the surface area of primary dendrites, which averaged 37 μm². Thus even in the extreme (and unlikely) case that primary dendrites have no spines, the surface area of a primary dendrite is still larger yet the distal dendrites produce larger responses to uncaged glutamate. By process of elimination, we conclude that the spatial variations in glutamate responsiveness arise from heterogeneity in the distribution of glutamate receptors. We therefore conclude that the density of glutamate receptors must increase with distance along the dendrites of both pyramidal neurons and interneurons and the same must be true for GABA receptors in pyramidal cells but not interneurons.

Whether these receptors are synaptic or extrasynaptic cannot yet be resolved, although it is likely that synaptic receptors dominate, at least on dendrites. Two extreme cases can be considered: the photoreleased transmitter could selectively activate synaptic receptors or could selectively activate extrasynaptic receptors. If only synaptic receptors were activated and the density of synapses is constant along the length of the dendrite (Trommald et al. 1995), then the relevant parameter is the length of dendrite exposed to transmitter rather than the area of dendrite. In this case, because the width of the light spot is the same in all regions, then the relevant parameter to measure is the absolute magnitude of currents generated by uncaged transmitter. We measured this and found substantial spatial gradients of responses for glutamate (Fig. 2A) and modest gradients for GABA (Fig. 5A) in apical dendrites of pyramidal neurons. We also observed marked differences in the distribution of glutamate and GABA responses in interneurons (Figs. 7A and 9A). In the case where only extrasynaptic receptors are activated, then the relevant parameter is the area of dendrite exposed to transmitter. We calculated the areas of dendrites exposed to transmitters and used these to determine the density of transmitter-induced currents. These measurements also indicated similar (or even steeper) gradients of transmitter responsiveness in these cells (Figs. 3C, 5C, 7B, and 9B). Thus both types of analysis we performed revealed qualitatively similar gradients of responses to glutamate and GABA, independent of whether the receptors for these transmitters are located at synapses or are extrasynaptic.

Except for somatic glutamate responses, which are produced by extrasynaptic receptors, we suspect that the other responses that we observed arise from activation of synaptic receptors. Assuming that this is the case, our measurements cannot determine whether the high neurotransmitter receptor density in distal dendrites is due to a larger number of receptors per synapse or due to a higher number of synaptic contacts in this region. At present, there are few and contradictory measurements of the relative distribution of synapses along the dendritic tree of hippocampal neurons. As mentioned above, spine density is thought to be uniform along the length of pyramidal cell dendrites (Trommald et al. 1995); if spines are proportional to the number of synapses, then synapse density also should be uniform. Consistent with this, Harris et al. (1992) report a uniform number of synapses along selected regions of the apical dendrites of pyramidal neurons in stratum radiatum, although Anderson et al. (1980) indicate an increasing density of synapses along the length of these same dendrites. If synapse density is indeed uniform along pyramidal cell dendrites, then the higher density of functional receptors that we observed is due to a higher density of receptors per synapse.

In summary, regardless of whether synaptic or extrasynaptic receptors were being activated, our results point to a gradient in the distribution of these receptors. These gradients are likely to be very important for the biology of these neurons. The gradients have not been revealed previously because until now no technique permitted mapping of functional transmitter receptors in central neurons, except in cultured neurons where the distribution of synapses and receptors is necessarily altered by cell dissociation.

**Functional implications of differential receptor targeting**

The enhancement of glutamate responses in the distal dendrites of both pyramidal neurons and interneurons may provide a mechanism that allows neurons to boost excitatory synaptic transmission at distal dendrites. We propose that the gradients of increasing receptor density that we observed may help compensate for the electrotonic filtering properties of dendrites, allowing neurons to accurately code synaptic strength in
a location-independent manner. Evidence of the efficacy of this mechanism is our observation that electrical currents recorded at the cell body were uniform despite the fact that the glutamate was delivered to dendritic sites that varied over hundreds of micrometers (Figs. 2A and 6A). This is strikingly reminiscent of the results of Jack et al. (1981), who found that electrical responses recorded from the cell body of spinal motor neurons were constant in amplitude even when synapses were activated at varying electrotonic distances along the dendrites. Variations in receptor density of the sort we have observed could account for such results and could also account for the observation that the amplitude of excitatory field potentials is independent of the location of the synaptic input in hippocampal pyramidal neurons (Andersen et al. 1980).

Our results are complimented by recent findings that voltage-gated ion channels also are nonuniformly distributed along the length of pyramidal cell dendrites (Magee 1998). The density of these channels also seems highest in the most distal dendrites and decreases spatial filtering by the dendrites (Cook and Johnston 1999; Magee 1998, 1999). Perhaps the mutual gradients of neurotransmitter receptors and voltage-dependent ion channels in dendrites work together to ensure fidelity of synaptic impact independent of synapse location. Previous work in cultured neurons suggests that AMPA receptors are clustered (Craig et al. 1994; Xia et al. 1999) and that some excitatory synapses contain only NMDA receptors (Gomperts et al. 1998; Liao et al. 1999; Petralia et al. 1999). Our results extend these observations by indicating longer-range gradients in receptor targeting. Such targeting may allow neurons to fine-tune individual synapses in response to changing synaptic input (Carroll et al. 1999; Shi et al. 1999) or to other signaling events.

Our findings that both GABA and glutamate receptors are present throughout pyramidal neurons imply that synaptic excitation is opposed by inhibition along the entire length of the dendritic arbor of these cells. Because inhibitory inputs limit the spread of excitatory signals, this arrangement of GABA receptors may confer local spatial control on incoming synaptic signals in pyramidal neurons. For example, long-term potentiation of their excitatory synapses can spread some distance (Schuman 1997), and local inhibition may help limit its range. The majority of interneurons do not project from the hippocampus (but see Ceranik et al. 1997) and perform circuit functions that are quite distinct from those performed by pyramidal neurons. We have found that the distribution of neurotransmitter receptors on these interneurons also differs from that of pyramidal cells. Our observation of a paucity of GABA receptors on the distal dendrites of these neurons suggests that synaptic excitation in distal interneuron dendrites is unopposed by synaptic inhibition and, therefore that local control of excitation is less critical for these cells than for glutamatergic pyramidal neurons.

In summary, the gradients of transmitter responsiveness we observe suggest that neurons target receptors to dendrites in a location-dependent manner. Similar spatial variations in the density of voltage-gated ion channels in these neurons (Cook and Johnston 1999; Magee 1998, 1999) suggest that regional variations in channel distribution may provide a general mechanism for preferentially regulating the efficacy of synaptic transmission. Future experiments can further exploit local photolysis of caged neurotransmitters to localize even more precisely specific receptor subtypes and to investigate the mechanisms responsible for receptor targeting.

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